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# Global genome-centric metatranscriptomics unravels food webs in complex microbial communities

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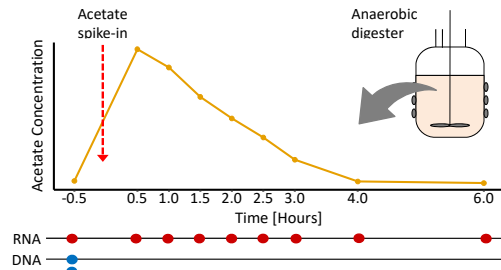
## Introduction

Advancements in high-throughput sequencing have led to an increasing amount of time series studies, investigating the temporal variation of microbial communities across multiple environments. However, the integration of this vast amount of data with high-quality metagenome-assembled genomes (HQ-MAGs) are lagging behind. We introduce a simple, quantitative framework linking MAGs to functional response and food webs.

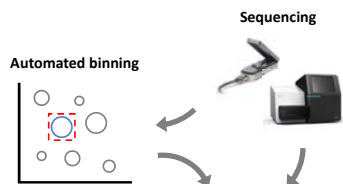
## Objective

Develop a genome-centric approach to characterize and quantify the activity of a complex microbial community using metatranscriptomics.

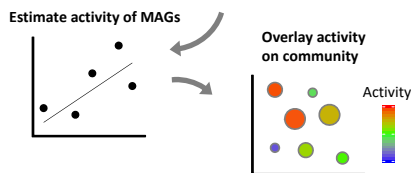
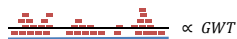
## Methods



**Experimental design** | A continuous stirred tank reactor was inoculated with sludge from an anaerobic digester. After a starvation period the reactor was stimulated with an acetate spike-in. Metagenomes for automated binning were obtained before spike-in and metatranscriptomes were sampled in a regular interval up to six hours after stimulation.



**Genome-wide transcription (GWT)**  
"Median gene expression per MAG per sample"



**Genome-wide expression analysis** | DNA samples were short-read sequenced and mapped to a Nanopore-only assembly for automated binning into MAGs. RNA samples were short-read sequenced and mapped directly to MAGs to estimate genome-wide transcription (GWT). Activity were computed as the cross-correlation between GWT and acetate concentration measurements across time-points, with a lag-time of one. The activity of each MAG were overlayed on the differential abundance plot to identify responders to acetate.

## Want to try it out for yourself?

- **mmgenome2** – [github.com/KasperSkytte/mmgenome2](https://github.com/KasperSkytte/mmgenome2)
- **mmtravis** – [github.com/TYMichaelsen/mmtravis](https://github.com/TYMichaelsen/mmtravis)

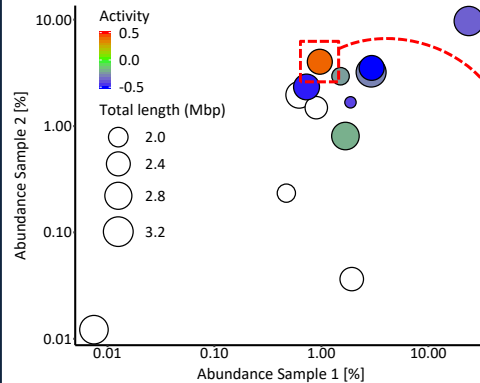
## Conclusion

Overlaying transcriptomics data on high quality draft genomes enable fast detection of reactive members in a microbial community.

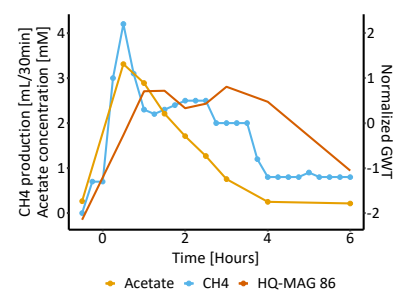
## Case-study: One HQ-MAG is responding to acetate

### A) 13 HQ-MAGs\*

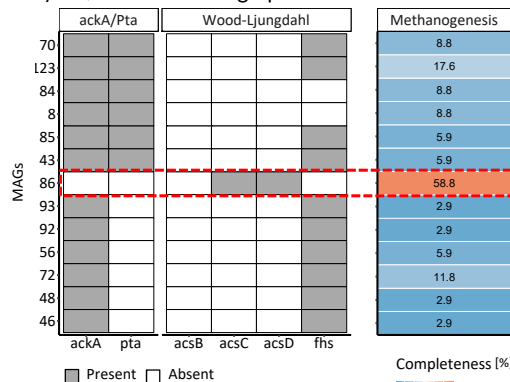
(completeness > 90%, contamination < 5%, 23S/16S/5S > 0, tRNA > 18)



### B) HQ-MAG 86 has positive response to acetate



### C) HQ-MAG 86 has high potential for acetate consumption



**Targeting active members in the community** | A) shows the differential abundance of all HQ-MAGs in two DNA samples taken half an hour before acetate spike-in. Each point represents a HQ-MAG scaled by size to its total length and coloured by the cross-correlation with acetate concentration. Insufficient data to compute GWT estimates are coloured white. B) one HQ-MAG (no. 86) has a positive cross-correlation and the GWT estimate for each time-point is plotted together with operational data, constituting the acetate concentration and CH4 production. C) all HQ-MAGs were screened for presence/absence of essential genes for acetate metabolism and completeness for the methanogenesis pathway.

\* Bowers R.M., Kyrpides N.C., Stepanauskas R., et al. (2017). *Nature Biotechnology*, 35(8), 725.

**GWT analysis:**  
HQ-MAG 86 responds to acetate

**Genomic analysis:**  
HQ-MAG 86 has high potential for acetate consumption

**GWT is validated!**

## Perspectives

In-depth analysis of HQ-MAGs using time series and correlation network analysis will enable the reconstruction of food webs and interrogate causal drivers for shifts in bacterial activity to identify novel keystone species. In addition, detection of genome-wide patterns as gene clusters and key driver genes will map out the biological functionality and potential of individual community members.

